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CLASSIFICATION OF CIRCULATING LEUKOCYTES
IN THE NORMAL MEXICAN BURRO

by

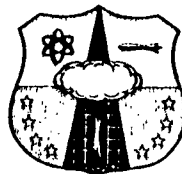
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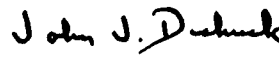
ABSTRACT

The morphology of circulating leukocytes of the normal burro is reported. The granulocytic and agranulocytic series are described and compared in part to those of the horse. Lymphocytes are divided into small, medium, large transitional, and plasma cellular types. Occasional cell types found in blood smears from the normal burro are described.

PUBLICATION REVIEW

This report has been reviewed and is approved.


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1. INTRODUCTION.

Series of tests using the burro (Equus asinus asinus) were designed to measure hematological effects produced by bilateral cobalt-60 irradiation to the region of the brain. The burro was chosen as the experimental animal because it is docile, larger than man, has a body volume near that of man, carries vital organs relatively the same distance from the ground as man, has a relatively long life span, is adaptable to variable climates, is easily handled, is easily maintained, and is excellent for repeated blood sampling.^{1,2} Later experiments at the Nevada Proving grounds (AEC) and the work of Trum and Rust and their co-workers have shown that the burro manifests neurological alterations at lower doses than other species; therefore, this animal appeared to be ideally suited for studying neurological changes attributable to ionizing radiation injury since these changes occur below the LD 50/30. Reversible or irreversible brain changes producing neurological symptoms might be associated with peripheral blood alterations; thus correlative values may exist in peripheral blood changes and neurological symptoms as noticed in the burro irradiated to the brain with small dosages of gamma radiation.

The burro has been used extensively in hematological studies following whole-body ionizing radiation exposures.¹⁻⁷ The normal hematological parameters of the Southwestern burro have been reported.⁸ The detailed cellular morphology of the peripheral blood of the normal burro has not been described, although color charts have been published.⁹

This preliminary report describes the cellular morphology of the leukocytes found in the peripheral blood of 46 normal adult male burros ranging in age from 3½ years to 8 years. It is submitted because of an increased interest in use of large animals in radiation research and so that it may serve as a source of data for such studies in which comparisons of normal data are desirable.

2. MATERIAL AND METHODS.

Blood samples obtained from the jugular vein were used for smearing two slides. The edge of a 75x25mm polished-edge No. 1 microslide was used to streak the blood. The slides were air dried; although in damp, cold air a hair dryer was used. The slides were stored in a standard slide box with separated compartments. The unstained slide appearing to have the best smear was

stained with modified Wright's stain.¹⁰

The differential cell counts were made in areas of the smears that contained all cell types, free from rouleau formation, and smeared to a one-cell thickness within a 20X field. These areas were not easily found because of the tendency of burro blood to undergo rouleau formation. For the most part, this area was posterior to the tongue of the smear and in the area that colored light was reflected. On each of 2,990 slides, 100 white cells were counted in determining their type.

3. RESULTS AND DISCUSSION.

To reduce technical error or poor distribution, careful attention to the technique of smearing and staining the blood film was of paramount importance.¹⁰ When poor smearing resulted, it was necessary to stain both slides. The inherent tendency of the lymphocytes to fall out in the middle, and the granulocytes to fall out on the outer edge of a smear, was apparent even in slides obtained with good technique. The eosinophils and the heavier cells had a tendency to fall off first in the smear.

Poor smearing and staining techniques having been avoided as much as possible, a consistent method of counting cells had to be devised. In the attempts to get a differential cell count for a true uniform distribution, the battlement, edge count, and the cross-sectional methods were tried. These methods had been proposed as helpful, but did not eliminate the errors due to nonuniform distribution of the cell types.

At least 400 cells must be counted before the results of a differential cell count may be considered reliable within 7.5 percent.¹² A series of 100 cell differentials observed under the given criteria presented a similar variance.

The metamyelocyte and less mature cells of the granulocytic developmental series of the burro are identical to those seen in the horse. These cells in the peripheral blood of the burro are not as rare as in the horse.

The cytoplasm of the band neutrophil is similar to that of the mature neutrophil. The nuclear chromatin is finely dispersed. The nucleus varies from long oval to horseshoe shape. If the constriction of the nucleus is more than three-fourths of its diameter, the cell is classified as a mature neutrophil. (It is necessary to put this restriction on the nucleus, because over one-half

of the neutrophils would be band neutrophils, if the classical description of band neutrophils is followed.) Some nuclei are twisted on themselves, giving the impression of segmentation. Because of the fine chromatin pattern, these cells are classified as band neutrophils.

The segmented neutrophil of the burro is quite similar to that in the horse. Numerous minute acidophilic granules are observed in the cytoplasm. Light green to blue-gray irregularly shaped fragments are found in the cytoplasm. The color, shape, and location of the fragments are consistent with "Dohle bodies." Their significance is unknown.¹³

Large triangular clumps of chromatin are attached to the indistinct nuclear membrane. These clumps are set off by clear, almost linear areas of parachromatin. Smearred chromatin occupies the remaining portions of the nucleus. Usually three to five lobes are present. These may be clearly connected by a condensed thread of chromatin; or if gathered on one another, indistinct separations occur, and a piled appearance results.

The eosinophil of the burro is similar to the eosinophil of the horse. The cytoplasmic granules are usually tightly packed, nonuniform in size and brightly eosinophilic. The cell has a red raspberry-like appearance because the cytoplasmic membrane conforms to the outpocketing of the granules. These granules obscure other organelles that may be present in the cytoplasm, but occasionally a light blue cytoplasm can be observed between them.

The nucleus usually has two lobes, but there may be only one or as many as four. The nuclear detail is obscured by the granules for the most part. Slight precipitation of chromatin occurs along the nuclear membrane. The chromatin-parachromatin separation is indistinct, thus giving the impression of smearing.

The basophil is identical to that in the horse. The cytoplasm contains either tightly packed or loosely scattered, irregularly shaped, strongly basophilic granules which usually partially obscure the nucleus which is frequently bilobed and appears smudged.

The small lymphocyte is the smallest of the lymphocytes, and seldom, if ever, divides.¹⁴ The cell membrane tightly encircles at least two-thirds of the nucleus which is usually round and characterized by heavy, chromatin

clumping. The typical nuclear halo is not usually observed in the small lymphocyte. The cytoplasm may have a mottled, hyaline, or homogeneous basophilia or it may be quite clear with azurophilic granules.

The medium lymphocyte approximates the size of the mature neutrophil. The cytoplasm can be basophilic and coarse, almost granular, or light blue, having a nonheterogeneous appearance. Azurophilic granules may be found. A clear nuclear halo is usually observed. The nucleus is generally round to oval in shape, but may be bean shaped, rectangular, or may have definite fissures. The nuclear margin is well defined. The chromatin and parachromatin are not clearly separated. There may be large chromatin clumps dispersed in a smudged chromatin background. Nucleoli are not observed, except in cells that have been spread out excessively. The nucleus is definitely mature.

The cell membrane of the large lymphocyte is delicate. This cell is larger than the mature neutrophil and assumes an oval or circular shape on the smear. The cytoplasm is light blue-green, and appears granular although no granules or azure dust can be identified. There may be small circular clear areas in the cytoplasm. In some cells, the cytoplasm appears to be divided into two zones. The outer zone is homogeneous and light blue-green; the inner zone is more heterogeneous and basophilic. The nucleus is large, round to oval and has a distinct nuclear membrane. It has a medium coarse chromatin structure. If the cell has a fine chromatin nuclear structure and basophilic cytoplasm, it is comparable to the prolymphocyte of Ferrata,¹⁵ and the prolymphocyte described by the Committee on Nomenclature.¹¹

The hematopoietic reticular cells and reticular lymphocytes are placed in the immature lymphocyte group. This group carries no criteria other than cells that are precursors of lymphocytes.

The monocyte is sometimes difficult to distinguish from the large lymphocyte.¹⁴ The cytoplasm varies from a light slate-gray to a light blue. Typical azure dust, noted in monocytes of other species, is observed. The nuclear membrane is delicate. The chromatin and parachromatin are quite distinct from each other. The fine chromatin structure is preserved. Small, discrete, delicate clumps of chromatin are scattered through the nucleoplasm.

The plasma cell is identical in morphology to those of other species.

The plasma cellular lymphocyte varies in size between the medium and the small lymphocyte. The cytoplasm is strongly basophilic. There is the impression that small acidophilic granules are present. The typical plasma cell "hof" is not seen, although the lymphocytic nuclear halo is usually discernible. The cell membrane is in the association with one-third of the eccentrically placed nucleus. The plasma-cellular lymphocyte nucleus has a radkern appearance, but does not appear as coarse as the smaller, more compact plasma cell nucleus. Downey¹⁶ found a similar cell in nine cases of benign lymphocytosis accompanied by adrenal hyperplasia. These cells showed plasma-cell characteristics and were described as "abortive plasma cells."

The plasma cell precursor attains a size comparable to the large lymphocyte. The narrow rim of cytoplasm has a strong basophilia and a granular appearance. There may or may not be a "hof." The large round nucleus is somewhat eccentrically located. Its membrane is extremely delicate. Discernible nucleoli are covered with finely dispersed chromatin.

Immature plasma cells are not always easily distinguished from the more basophilic precursors of lymphocytes. Studies indicate there are lymphatic and myeloid types of plasma cell.¹⁵ The transformation of lymphocytes to large, basophilic cells resembling hematopoietic reticular cells has been acknowledged. This multipotentiality of the lymphocyte has been demonstrated.¹⁷⁻²¹ Sundberg¹⁵ states,

Plasmoblasts, hematopoietic reticular cells, and reticular lymphocytes are morphologically distinguishable from one another, but often their similarities are greater than their differences. The most remarkable differences are the intensity and opacity of cytoplasmic basophilia, the assumption and retention of an eccentric nucleus, and the relatively discrete clumping of the nuclear chromatin of the immature plasma cell.

Somewhat similar cells were found by Schulze²² in a case of agranulocytosis in a horse. These cells had intensive blue staining cytoplasm with vacuoles and a fine azurophilic granulation. The intensely staining oval nucleus had agglomerations of clumps of chromatin.

The transitional lymphocyte resembles the true monocyte very closely. It differs, however, in several aspects. The cytoplasm is more reddish and gives the impression of much larger granulation, or it may be strongly basophilic with no demonstrable azure dust. The nucleus may be lobulated, bean shaped, or may show increased irregularities. It has large clumps of chromatin and the distinction between the parachromatin and chromatin is not as sharp as in the monocyte. Various degrees of cytoplasmic and nuclear changes are observed. The term "transitional" is used because the cell resembles a number of cell types. Lymphocytes are known to transform into histocytes,²³ macrophages,²⁴ and plasma cells.^{15, 19, 21} There is no attempt to follow this series of cells further than classifying them as transitional cell types.

The following description of cells is intended to show cell types occasionally found in the normal smears during this study. They were not found in every animal examined. However, they were observed often enough to be mentioned.

The binucleate lymphocyte is identical to the medium lymphocyte with the exception of the nuclear structure. Binucleate lymphocytes are said to have undergone amitotic division, but this might be due to deranged mitotic division.²⁵ These cells are nonspecific and may occur during antibiotic therapy and in damaged lymphoid tissue.^{25, 26}

Occasionally, cells are found in the stained smears resembling leukocytoid lymphocytes¹⁹ or Downey type II lymphocyte.¹⁶ Dougherty²⁷ found that the type II lymphocyte was not specific for infectious mononucleosis and that it was produced by nonadrenocortically mediated response to stress. He states, "The numbers and types of circulating lymphocytes reflect the degree of balance existing between adrenocortically mediated and nonadrenocortically mediated responses to stress stimuli."

Only on very rare occasions are megakaryocytes found in the peripheral

blood smears. Just as rare were eosinophilic cytoplasmic inclusions in the medium lymphocyte and the monocyte.

Cytoplasmic budding and pyknosis or karyorrhexis are observed in the lymphocytes. Frank and Dougherty²⁷ assume these are due to ACTH and cortisone susceptibility of the mouse lymphocytes. Neither pyknosis nor karyorrhexis is associated with the number of smudged leukocytes. Such cells found in stained human blood smears have little or no direct relationship to dead cells.²⁹ A small and constant number of dead leukocytes are found in normal blood.²⁹

4. SUMMARY.

One hundred differential cell counts were made from 2,990 different peripheral blood smears from 46 normal adult male burros. The granulocytic series is found to be similar to the horse. The mature agranulocytic series, composed of small, medium and large lymphocytes, does not differ from that of the horse. Hematopoietic reticular cells and reticular lymphocytes are categorized in the immature lymphocytic group. The plasma cellular lymphocyte, plasma cell precursor, and transitional lymphocyte are considered to be expressions of the multipotentiality of the lymphocyte. Binucleated lymphocyte, Downey type II lymphocytes, and megakaryocytes are occasionally observed in peripheral blood smears.

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